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# Vancomycin-Resistant *Enterococcus faecium* Strains Isolated from Community Wastewater from a Semiclosed Agri-Food System in Texas

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**Vancomycin-resistant *Enterococcus faecium* strains (VRE) were isolated from human wastewater but not swine fecal waste from a semiclosed agri-food system in Texas. Forty-nine VRE isolates possessed *vanA*, and one possessed *vanB*. Twenty-one pulsed-field gel electrophoresis types were identified and segregated into three groups. There was evidence of clonal dissemination among geographically separated sites.**

Many factors are involved in the global dissemination of vancomycin-resistant enterococci (VRE), as evidenced by the emergence of *Enterococcus faecium* complex 17 (22). VRE are highly prevalent among hospitals in the United States (2, 6) and have been reported from hospital wastewater but have not been isolated from livestock or any healthy human population (4, 7, 10, 16, 18, 20). There has been speculation that VRE exist among the general population in the United States and that more should be done to identify community carriage of VRE so control measures can be instituted (14).

The *vanA* gene cluster, which confers high-level resistance to vancomycin and teicoplanin, is located on transposon Tn*1546* and consists of seven genes: *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* (1). Genotypic characterization of mobile genetic elements, such as Tn*1546*, can be done independently from chromosomal analysis of individual clones, thus providing information on the transmission of the mobile element (5, 19, 21).

The purpose of this study was to characterize the VRE isolated from human wastewater effluents located within an integrated semiclosed agri-food system in Texas. This is the first known report of VRE shedding from a largely nonclinical community population in the United States.

**Study population.** The study population and sampling procedures have been previously described (17). Briefly, the study population consisted of humans and swine at 18 geographically separated farm and non-farm sites in Texas. Thirteen sites housed both swine and human cohorts. Within the study group, swine worker facilities housed 3,000 people, while non-swine worker facilities housed 18,000 people. Wastewater effluent from swine worker and non-swine worker dormitories drained into a central lift station and proceeded to a wastewater treatment plant. Each of the seven facilities that yielded VRE had its own separate wastewater treatment plant, such that VRE dissemination between facilities via wastewater would not have been likely. Swine fecal samples consisted of composite feces and lagoon samples. The swine breeding program was self-sustaining, and swine were not moved between farm sites. Those used for food production were moved to a single slaughter facility, where they were processed and consumed within the system. Approximately 50,000 swine were slaughtered each year.

**VRE.** From October 2002 to August 2004, 1,252 human effluent and 1,270 swine fecal or effluent samples were analyzed for enterococci. VRE were isolated on M-enterococcus agar (Becton Dickinson, Sparks, MD) containing 20 μg/ml vancomycin and were incubated for 48 h at 45°C. Isolates from M-enterococcus agar were identified as *E. faecium* by the API 20 Strep system (bioMerieux, Hazelwood, MO) and PCR as described by Jackson et al. (11).

The MICs of the antimicrobials were determined by broth microdilution according to methods described by the Clinical Laboratory Standards Institute (3). Fifty enterococci that demonstrated high-level resistance to vancomycin (≥512 μg/ml) were isolated from human wastewater. No VRE were isolated from swine samples (Table 1). All VRE were susceptible to daptomycin, linezolid, and quinupristin-dalfopristin. Ninety-two percent of the VRE exhibited macrolide and lincosamide resistance. Seventy-four percent of the VRE isolates exhibited high-level resistance to gentamicin (≥1,024 μg/ml).

TABLE 1. Total number of samples selectively screened for all enterococci and VRE by year, host species, and sample origin

| Host species | Sample origin | No. of samples by yr[a] | | |
|---|---|---|---|---|
| | | 2002 | 2003 | 2004 |
| Human | Swine worker | 0 | **21**/223 | **5**/211 |
| | Non-swine worker | 0 | **1**/79 | **11**/369 |
| | Mixture[b] | **3**/27 | **5**/171 | **4**/172 |
| Swine | Composite fecal | 47 | 77 | 809 |
| | Influent | 16 | 18 | 303 |

[a] Boldfaced numbers are the number of VRE isolates obtained from the sample groups.
[b] Mixture means samples composed of non-swine worker and swine worker wastewater or samples taken from the central core facility or hospital facility that does not include swine workers.

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TABLE 2. Geographic location, PFGE type, and date of VRE isolate recovery[a]

| Isolate (PFGE type) and date (mo/day/yr) of recovery at location (facility type): | | | | | | |
|---|---|---|---|---|---|---|
| TX1 (farm affiliated) | TX2 (farm affiliated) | TX3 (farm affiliated) | TX4 (farm affiliated) | TX5 (farm affiliated) | TX6 (hospital) | TX7 (CCF) |
| 14 (A) 6/6/03 | 22 (L) 6/6/03 | 25 (C) 6/6/03 | 143 (A) 7/1/03 | 301 (T) 4/9/04 | 373 (N) 5/3/04 | VB (S) 10/4/02 |
| 15 (A) 6/6/03 | 23 (B) 6/6/03 | 29 (A) 6/6/03 | 144 (A) 7/1/03 | 700 (F) 8/6/04 | **593 (N)** 6/18/04 | **653 (R)** 7/12/04 |
| 16 (D) 6/6/03 | 24 (A) 6/6/03 | 60 (A) 6/6/03 | 145 (A) 7/1/03 | 701 (G) 8/6/04 | **594 (P)** 6/18/04 | **654 (Q)** 7/12/04 |
| 17 (D) 6/6/03 | 50 (A) 6/6/03 | | 146 (A) 7/1/03 | 702 (E) 8/6/04 | 749 (K) 8/16/04 | |
| 18 (A) 6/6/03 | 51 (A) 6/6/03 | | 147 (A) 7/1/03 | 703 (G) 8/6/04 | 751 (J) 8/16/04 | |
| 19 (A) 6/6/03 | 223 (A) 7/24/03 | | 246 (U) 4/9/04 | | | |
| 54 (A) 6/6/03 | 224 (A) 7/24/03 | | **261 (H)** 7/24/03 | | | |
| | 225 (A) 7/24/03 | | **262 (H)** 7/15/03 | | | |
| | 226 (A) 7/24/03 | | **280 (H)** 7/31/03 | | | |
| | 228 (A) 7/24/03 | | **282 (H)** 7/29/03 | | | |
| | 336 (A) 4/20/04 | | **284 (H)** 7/29/03 | | | |
| | 337 (A) 4/20/04 | | 672 (M) 8/6/04 | | | |
| | 338 (A) 4/20/04 | | **9561 (O)** 10/4/02 | | | |
| | 9674 (I) 10/4/02 | | | | | |

[a] Farm affiliated, facility with both a swine farm and human dormitory; human hospital, no swine; CCF, central core facility for human personnel. Isolates in boldface do not have IS*1251*.

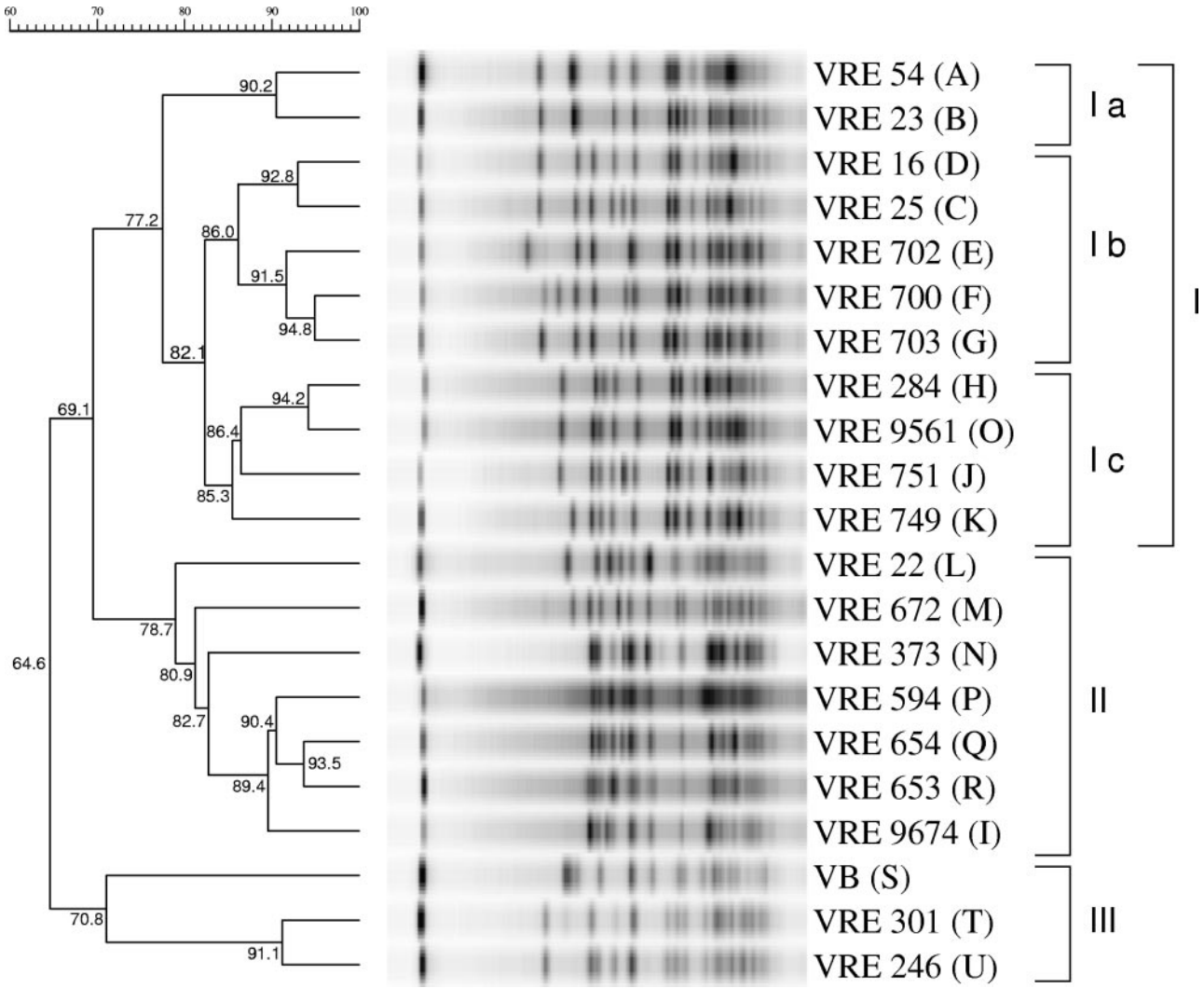


FIG. 1. Dendrogram illustrating relatedness of 21 PFGE types. The bar indicates the percent similarity coefficient.

PCR genotyping of *vanA*, *vanB*, and *vanC* VRE has been previously described (8). Forty-nine *E. faecium* isolates possessed the *vanA* gene cluster, and one, VB, carried the *vanB* gene cluster. All VRE exhibiting high-level resistance to gentamicin were shown to possess the bifunctional gene (*acc6′-aph2*) that confers high-level resistance to aminoglycosides. Forty-three VRE isolates (86%) possessed the rRNA methylase gene (*ermB*) that confers macrolide, lincosamide, and streptogramin B resistance.

The use of PCR fragment length polymorphism analysis to detect intergenic insertion sequences has been previously described (13, 19). PCR analysis of the intergenic regions of Tn*1546* was done to detect heterogeneity among the 49 *vanA* isolates. Two types of Tn*1546* element were identified. Thirty-nine VRE carried IS*1251* in the *vanSH* intergenic region (Table 2, isolates not in boldface), and no other polymorphisms were detected. No insertion sequences were detected in the second type of Tn*1546* element, carried by 10 isolates. To date, IS*1251* has only been identified in VRE isolated from hospitals in the United States (9, 12, 13, 21). A number of polymorphic Tn*1546* types have been identified among VRE in the United States (5, 9, 12, 13, 21); it would be of interest to know how many of the types are endemic to public hospitals in Texas.

All isolates were examined by pulsed-field gel electrophoresis (PFGE) as previously described (15). Twenty-one PFGE types, arbitrarily designated A to U and represented by three groups (groups I, II, and III), were detected among the 50 VRE isolates (Fig. 1). Group I included 39 isolates (78%), the most predominant being PFGE type A ($n = 23$). All type A clones were indistinguishable by antimicrobial susceptibility profile and molecular analysis. Type A clones were disseminated among four geographically separated swine farm locations (TX1 to TX4) (Table 2). The distance between farm sites ranged up to 200 km. Twenty (87%) of the type A clones were isolated in June and July of 2003; however, three were isolated in April 2004.

All group I VRE were isolated from farm facilities with the exception of VRE 749 and 751 (85.3% similarity coefficient), both of which were isolated from a hospital facility (TX6) (Table 2) in June 2004.

At TX1 and TX3, VRE were only isolated once. VRE persisted through the duration of this study at TX2 and TX4. The greatest degree of heterogeneity among VRE was observed from those isolated at TX4. The presence of related VRE PFGE group I and II isolates from 2002 to 2004 may suggest the ability of VRE to persist in the study community or in community wastewater over extended periods of time. The presence of identical VRE clones (PFGE type A) at multiple geographically separated farm sites demonstrates the occurrence of clonal VRE dissemination.

All human subjects were enrolled at the central core facility (TX7) and were subsequently assigned living quarters. Individuals who had been in the system for long periods of time were selected for farm work; once assigned, there was very little movement of farm workers among farm sites. There was virtually no movement of non-farm workers among any sites. VRE carrier status was not known at the time of entry; however, none of the current farm workers would have entered the system during the time period of this study. As in any community population, individuals may have need of hospital (inpatient) or dispensary (outpatient) services from time to time. Each site is equipped with a dispensary. Because the medical histories and documentation of movement among workers were not available, the mechanism for dissemination to geographically separated sites is unknown. However, additional population studies and genetic analyses of the VRE in this study combined with VRE from hospital reservoirs in Texas could conceivably trace the origin of these isolates.

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